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A Hydrogen-Bond Stabilized Mechanism of Oxygen Evolution in Photosystem II: A Proposed Computational Experiment

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Abstract: The ability of plants to take in water and release oxygen into the atmosphere is crucial to the survival of life on Earth. During photosynthesis, water is oxidized to O_2 (dioxygen) at the Oxygen Evolving Complex (OEC) of Photosystem II. Structurally, the OEC resembles a box with an open lid, consisting of metal atoms (four manganese and one calcium) bridged by oxygen atoms. The mechanism of action of this complex, however, is not well understood. Various mechanisms have been proposed in recent years to explain how the OEC oxidizes water to dioxygen, but all of these mechanisms contain gaps and require further attention. I believe I have come across a previously unconsidered feature of the OEC that is essential for its function.

The oxidation of water (that is, the loss of electrons from the water molecule, resulting in its transformation to dioxygen) occurs primarily through H_2O's oxygen atom, where most of its electron density is located. The metal atoms of the OEC perform the oxidation. In the complex, each of these metal atoms is flanked by two oxygen atoms. I noticed that these two oxygen atoms are perfectly positioned to serve as hydrogen-bonding "docking sites" for the two hydrogens in a water molecule while the metal atom interacts with the water molecule's central oxygen atom. It is my belief that this interaction could be necessary to stabilize the water molecule as it is being oxidized. If true, it is likely that this stabilizing interaction is required for efficient water oxidation at the OEC, which has tremendous implications for the development of renewable energy technology – specifically, that including oxo bridges in the structure of synthetic water oxidation catalysts is necessary to design an efficient energy source whose only by-product is molecular oxygen. In this study, principles of physical and inorganic chemistry are applied to currently proposed OEC mechanisms to determine which is most favorable; a computational experiment will then be designed which could probe whether hydrogen bonding at the oxo bridges increases the efficiency of the OEC.

Introduction

The remarkable ability of plants to convert raw materials into a source of energy and release only oxygen as a by-product has captivated researchers for decades, and the race to emulate this process in a man-made catalyst has only become more urgent as legislators and the public call for more efficient sources of renewable energy. Glucose, the substrate for the energy-producing glycolysis process, is produced in the so-called dark reactions in plants; the energy source and reducing power behind the dark reactions are Adenosine Triphosphate (ATP) and Nicotinamide Adenine Dinucleotide Phosphate (NADPH), respectively, whose production is driven by the transport of electrons along a series of

carriers in the light reactions. The ultimate source of these electrons is water, which is split into protons and oxygen at the Photosystem II complex (PS II), located in the thylakoid membranes of chloroplasts. When 680 nm light strikes PS II, it becomes photoexcited, passing one of its electrons on down the chain of electron carriers, whose activity eventually generates both ATP and NADPH. This process inevitably leaves PS II with an electron hole, so a consistent supply of electrons to PS II is required for glucose synthesis to continue. PS II obtains these electrons through the oxidation of water to molecular oxygen (O_2), which occurs at the Oxygen Evolving Complex (OEC), a metal-oxo cluster of the general formula $CaMn_4O_5$.

X-Ray Diffraction (XRD) studies of the OEC form the basis of the current understanding of its structure. The first crystal structure to resolve the cubane OEC unit complete with μ-oxo bridges between the metal atoms was solved by Ferreira et al.;[1] this cubane structure was further refined by the XRD data of Umena et al.[2], and it is on this structure that the present work is based. Unfortunately, the position of the water molecules remains uncertain in these structures, and XRD is unable to detect protons, making it very difficult to ascertain exactly where and how the substrate water molecules interact with the complex. It is also extremely difficult to track this reaction at the molecular level *in vivo*, particularly the crucial O-O bond forming step, in which a final proton must be jettisoned and molecular oxygen formed. As a result of these complications, to this day no single mechanism has completely won over the bioinorganic chemistry community.

Aside from the structure of the OEC, several aspects of its activity are known with certainty and are required components of any proposed water-splitting mechanism. First, any acceptable mechanism must include Kok's S-State Cycle, the sequential production of four oxidizing equivalents from the OEC per molecule of O_2 released.[3] Kok's experiments involved exposure of isolated chloroplasts to flashes of light at regular intervals, with measurement of any oxidizing equivalents or O_2 released after each flash. Sequential oxidation of the complex by +1 following each of four flashes was observed, with O_2 being released after the final flash and the process beginning again. These results led Kok to propose a linear four-step mechanism involving five different manganese catalyst oxidation states ("S-States"), with release of a proton and an electron between each S state. In this cycle, return to the ground S_0 state is permitted by release of a molecule of O_2 and concomitant uptake of a new water substrate.

Kok's S-Cycle has been refined by further experimentation – namely, a 1:0:1:2 ratio of proton release across S-States has been accepted based on local electric field measurements[4,5], and involvement of the radicalized tyrosine residue Y_Z as a key redox cofactor has been confirmed[6] – but the basic premise remains intact. Generation of four oxidizing equivalents throughout the cycle is mandatory in a satisfactory mechanism. Additionally, it has been determined through $H_2{}^{18}O$ labelling experiments that two substrate water molecules bind at separate sites throughout the cycle[7], and that one of these waters is bound to the calcium atom[8]. An appropriate mechanism of action for the OEC must include these details as well.

Despite the numerous mechanisms proposed to explain how the OEC oxidizes water, to my knowledge no one has yet considered that the bridging O atoms may serve as hydrogen bond "docking sites" for the protons of a substrate water molecule. Such an interaction would stabilize each water molecule on the OEC at the appropriate S-States, potentially lowering its overall energy and thus providing a more favorable reaction dynamics pathway for the oxidation. If the proposed hydrogen bonding scheme is calculated to have an energy-lowering effect on the complex, it is quite possible that the energy reduction arising from this interaction permits the oxidation reaction to occur in the first place. Vrettos et al. have observed[9] that hydrogen bonding between the protons on the calcium-bound water and

nearby acceptors would weaken the Ca-O bond and sufficiently to allow for O-O bond formation in the S4 state; they propose a nearby histidine residue, D1-H337, and one of the O atoms in the complex to be the acceptors. It may be surmised that if both hydrogen-bond acceptors are part of the complex itself, as the bridging O atoms are, then this stabilizing effect would be amplified due to the shorter donor-acceptor distance.

The purpose of this paper is two-fold: (1) to conduct a minireview of currently proposed OEC mechanisms, in which the one which is most compliant with current data and the principles of inorganic and physical chemistry will be determined, and (2) to design a computational experiment to test whether hydrogen-bond stabilization of the substrate waters at the bridging O atoms is an important component of the OEC mechanism.

Review of Currently Proposed Mechanisms

In order to accurately determine the effect of the proposed hydrogen-bonding scheme on the energetics of the OEC water oxidation reaction, it was necessary to select a reliable, previously proposed mechanism to serve as a control. It is logical that the "most correct" currently proposed mechanism should serve as the control, to ensure that all aspects of this proposal are consistent with current data. The following previously proposed mechanisms were considered for use as a standard:

BUTTERFLY/DOUBLE-PIVOT MECHANISM

The first complete water oxidation mechanism to gain widespread approval was the Double-Pivot, or "Butterfly," mechanism, based on data from the Christou[10] and Dismukes[11] groups. In this mechanism, the substrate water molecules were assumed to bond to adjacent manganese (Mn) atoms in the complex, which protruded outwards from the complex, much like the wings of a butterfly (hence the moniker). Sequential oxidation of the substrate waters was believed to give rise to μ-hydroxo bridges between the Mn atoms, creating a cubane structure of the sort observed in the X-ray data; further oxidation of these bridges released the bridging O atoms as O_2, opening the "wings" and returning the catalyst to the S_0 state. This mechanism successfully explained the oxidation of two water molecules to yield one molecule of O_2, with the release of the four oxidizing equivalents along the way.

The "Butterfly" mechanism began to lose traction with chemists when ESI-MS isotope exchange measurements on synthetic analogues[7] yielded exchange rates for the μ-oxo groups which were much slower than the observed exchange rates in the actual OEC. To examine the validity of this claim, the structures of the six synthetic OEC analogues from the isotope exchange measurements were reviewed for this study. In the context of this proposal, it is important to note that only one of these structures contained additional metal-oxygen-metal motifs beyond the one assumed to break open during the oxidation; even in this structure, all of the Mn atoms are coordinatively saturated, barring the approach of a substrate water to any of the additional bridging oxygens. If the hypothesis stated in this paper is correct, then it is probable that the slower exchange rates observed for the synthetic analogues are a consequence of the lack of available hydrogen-bond "docking sites" on the models to the substrate waters. Without this stabilization, the free energy required for the reaction would increase relative to the actual OEC, prohibiting rapid exchange of the substrate water molecules. By providing this additional perspective, the hypothesis espoused in this paper – if correct – could bring the Butterfly Mechanism back into favor with the bioinorganic chemistry community.

Eventually, however, the Butterfly Mechanism was rejected as a basis for this study due to structure and geometry considerations. This mechanism involves binding of both substrate water molecules to

Mn atoms on the complex, and more recent isotope exchange data involving replacement of the complex-bound calcium (Ca) atom with strontium[8] highly favor binding of one of the substrate waters to the Ca atom. Even if the Butterfly Mechanism is adapted to involve binding of one of the waters to the Ca atom, the mechanism no longer fits with the known structure of the complex, as the crucial O-O bond forming step in the mechanism requires that the four participating atoms be arranged in a diamond-like fashion, with the two metal atoms connected by two μ-oxo bridges. Ca and the "dangling manganese" on which the slow-exchange water substrate is believed to reside[9,12] are linked by only one μ-oxo bridge, and this bridging oxygen is bonded to four metal atoms[1,2] (more than any other O atom in the complex), making its release as a component of O_2 unlikely. On account of these discrepancies, the Butterfly Mechanism was rejected as a standard against which to compare the proposed mechanism.

2+2 "DIMER OF DIMERS" MECHANISM[19]

This mechanism was based on a dimeric model of the OEC in which the subunits were positioned to hold the two substrate water molecules in close proximity to each other. Sequential oxidation of the substrates left one subunit of the dimer with a coordinated hydroxyl group and the other subunit with a terminal manganese-oxo moiety (Mn=O). The O-O bond forming step then occurs by deprotonation of the hydroxyl and associated attack by the resultant nucleophilic oxygen on the Mn=O, forming a transient peroxo species which dissociates into the original dimer and O_2. This mechanism has been more or less discarded as more recent structural data fails to support the dimeric structure[1,13], and it would hardly be worth a mention here except that it forms the basis for the remaining mechanisms discussed in this paper, which all invoke water or hydroxyl attack on a high-valent manganese-oxo group as the O-O bond forming step.

LIMBURG ET AL. MECHANISM[14]

This mechanism involves sequential oxidation of each water molecule, one on the Ca atom and one on the "dangler" manganese, until all that remains is a hydroxyl group coordinated to the calcium and a terminal oxo group on the manganese. Nucleophilic attack on the Mn=O by the hydroxyl forms the O-O bond and leaves room for two new waters to bind. The Cl^- ligand "tunes" the nucleophilicity of the -OH group throughout the different S-states.

This mechanism is not unreasonable; in fact, it has much in common with the mechanism which was eventually chosen as the basis mechanism for this study. However, this mechanism is based entirely on data from synthetic Ru=O and Mn=O structures designed specifically to react in this way. This is not overwhelmingly convincing in terms of the action of the actual OEC.

PECORARO ET AL. MECHANISM[15]

This mechanism is nearly identical to that proposed by Limburg et al., with the exception that the synthetic catalysts used were designed to have potential differences similar to those observed for the different S-states in the OEC. While this certainly provides more evidence for the hydroxide-attack-on-oxo hypothesis, it is still not particularly conclusive in terms of explaining the actual behavior of the OEC – rather, it just confirms that the synthetic compounds behaved as they were designed to. More experimentation on the OEC itself is required to propose a reliable mechanism.

DAU ET AL. MECHANISM[16]

This highly convincing mechanism is based almost exclusively on this group's X-Ray Absorption

(XAS) and Extended X-Ray Absorption Fine Structure (EXAFS) studies of the OEC itself. The reliability of this study stems not only from the group's choice to study the actual enzyme instead of an analogue, but also from the fact that X-Ray techniques are able to resolve both relative atomic positions and metal oxidation states. This mechanism, like several others which remain popular today, invokes a manganese-oxyl radical and a hydroxyl radical as the effectors in the O-O bond-forming step. This choice was arbitrary and is not a product of the data collected by Dau and colleagues, who admit that "it is presently unknown how the formation of a ligand radical (e.g. a Mn-bound oxyl radical) might affect the X-ray edge." Further, regarding all such mechanisms, it should be noted that Mn-oxyl and Mn-oxo are spin isomers and essentially resonant with each other (vz. Fig. 10 in ref. 16). The remainder of the mechanism is unaffected by this choice, and deliberation between the radical versus the nucleophile is outside the purpose of the study presented here.

While convincing in terms of oxidation state assignments, XAS is not a standalone technique when it comes to explaining complex mechanisms. Certain elements of the mechanism proposed by Dau et al. jar significantly with trusted data from other studies. For example, unable to conclude a specific subunit arrangement from their X-ray data, the group selected a dimer-of-dimers structure as the basis for their mechanism, which has since been discarded with the advent of more reliable data[1,2,13]. This mechanism also postulates the involvement of two Mn atoms as the oxidizing centers, a less-promising suggestion now that experiments involving replaced of the Ca atom with Sr argue strongly for an oxidizing role for calcium. While the X-ray data presented by Dau and colleagues serves well to inform future studies, newer data has come to disfavor the mechanism proposed therein.

VRETTOS ET AL. MECHANISM[9]

Vrettos and colleagues have proposed a good, sound mechanism based solidly on Electron Paramagnetic Resonance (EPR), X-Ray Near-Edge/Extended X-Ray Fine Structure Spectra (XANES/EXAFS), and $H_2{}^{18}O$ exchange data, in conjunction with theoretical predictions and model chemistry. Their mechanism also cites nucleophilic attack by a coordinated water molecule on a high-valent manganese-oxo moiety, albeit in greater detail than others that were reviewed. The cycle begins in the resting S_0 state, with one water ligated to Ca (based on the the observation that the process stalls at S_2 if Ca is removed) and the other bound to the least-oxidized Mn center. Oxidation states of +2, +3, +4, and +4 are assigned to the four Mn atoms based on EPR and XANES data, and a μ-OH bridge is suspected between the Mn(II) and Mn(III) atoms based on the low oxidation states of this pair. Deprotonation of this bridge with concomitant loss of an electron oxidizes the Mn(II) to Mn(III), bringing the cluster into the S_1 state. The two Mn(III)'s are oxidized sequentially to Mn(IV) centers in the $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ transitions, the latter occurring by transfer of an electron to the Tyrosyl Z radical ($Y_ZO\cdot$). Further oxidation to the S_4 state also involves $Y_ZO\cdot$ as the electron acceptor, a process which merits some discussion. In both the $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_4$ transitions, one proton from the Mn-bound substrate water is passed to $Y_ZO\cdot$ to Y_ZOH during oxidation of the Mn center, supported by prior results indicating that reduction of Tyrosine Z occurs at these exact points in the S-state cycle . This results in the following progression for the Mn-bound water: $Mn^{III}\text{-}OH_2$ (S_2) → $Mn^{IV}\text{-}OH$ (S_3) → $Mn^{V}{=}O$ (S_4). From the S_4 state, Vrettos et al. propose that the substrate water ligated to calcium attacks the Mn=O moiety, transiently forming a $O\cdot\cdot H\text{-}O\text{-}O\text{-}Mn^{III}$ linkage before release of the peroxo component as O_2. This mechanism of O-O bond formation and O_2 release was directly inspired by the mechanism of reversible O_2 binding in hemerythrin. Considering the lack of available data regarding this step in the mechanism, analogy to a similar enzyme seems to be a prudent choice. The fast-exchanging water is postulated to be bound to Ca and the slow-exchanging water to a Mn based on prior mass spectrometry

experiments measuring rate of $H_2{}^{18}O$ incorporation into the OEC. Other important features of the Vrettos mechanism include the involvement of a Cl^- cofactor in the oxidation (found on experiments demonstrating a stalled S-cycle when chloride is replaced with acetate), as well as hydrogen-bonding of the Ca-bound substrate water to one bridging oxygen and the nearby histidine residue D1-H337, discussed in depth later (see Theoretical Basis).

This mechanism was selected as the basis on which the proposed hydrogen-bonding scheme will be founded, owing to its incorporation of diverse biophysical data, computational results, physical constraints, and chemical logic into one "best" mechanism. This does not conclusively make it the right mechanism, and it can be improved upon, which is the objective of this paper.

Theoretical Basis

EXPERIMENTAL DESIGN

The OEC mechanism proposed by Vrettos and coworkers is consistent with a wealth of biophysical data and fundamental inorganic chemistry and serves as an excellent foundation on which to test the presently proposed hydrogen-bonding scheme. Keeping this underlying mechanism constant, the independent variable in the proposed experiment would be the arrangement of the hydrogen-bond network around the two substrate water molecules.

This will be a purely theoretical/computational experiment, given the extreme difficulty in isolating higher S-state intermediates from the cycle. The energy of the complex under the hydrogen-bonding scheme proposed by Vrettos et al. (hereafter "Vrettos Mechanism") will be calculated and directly compared to its energy under the hydrogen-bonding scheme proposed in this paper (hereafter "Proposed Mechanism") for the S_1 and S_4 states. As in any reaction dynamics pathway, and in nature in general, the *lower energy structure is more likely to exist*. If the structures under the Proposed Mechanism are associated with a lower energy than the Vrettos Mechanism intermediates, that would strongly suggest that hydrogen bond stabilization of the substrate waters at the μ-oxo bridges is required for thermodynamically favorable water oxidation at the OEC.

The structures to be compared are shown in **Figure 1**. In the S_1 state, Vrettos and colleagues ascribe no hydrogen-bonding network to the Mn^{II}-bound substrate water molecule, making calculation of its energy relatively simple; in the proposed mechanism, the Mn^{II}-bound water hydrogen bonds to the bridging oxides Mn^{II}-**O**-Mn^{III} and Ca-**O**-Mn^{III}. The Vrettos S_4 state involves hydrogen-bonding of one proton on the Ca-bound water to the Mn^{V}-**O**-Mn^{IV} μ-oxo bridge and the other to the nearby histidine residue D1-H337. In contrast, the proposed hydrogen-bond acceptors in the S_4 state are the μ-oxo bridges at Mn^{V}-**O**-Mn^{IV} and Ca-**O**-Mn^{IV} (the same bridging oxygens that the Mn-bound water interacted with before its oxidation to Mn=O). The proposed arrangements fall within the acceptable donor-acceptor angle for hydrogen-bonding[17] and could certainly stabilize the water substrate in a way that favors the removal of its protons.

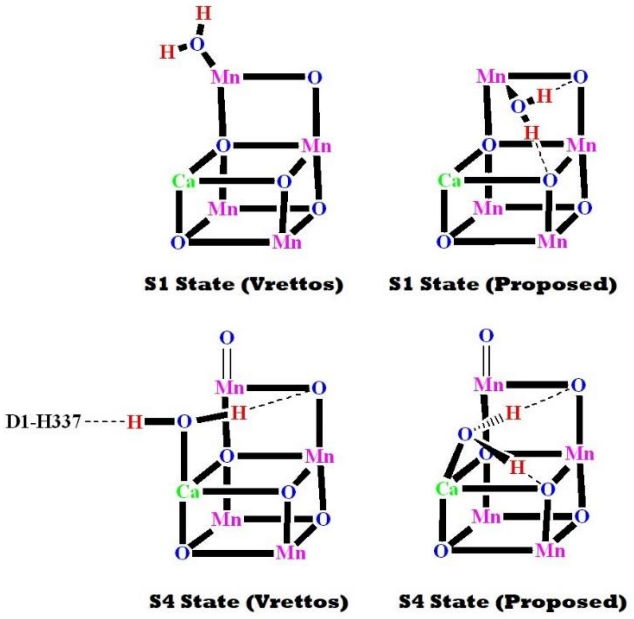

Figure 1: OEC hydrogen-bonding schemes for which energies will be calculated and compared.

METHODS

Density Functional Theory (DFT) has become the method of choice for calculating structures, energies, and interactions of large inorganic complexes[18] due to its ability to provide highly accurate results at minimal computational cost. The basis for all DFT methods is Hohenberg-Kohn Theorem, which states that all ground-state properties of a system depend only on its electron density[20]. Thus a system of interacting electrons moving in a real potential may be replaced by a fictitious system of non-interacting electrons moving in an effective potential *which has the same electron density as the real system*. The energy of the molecule/complex can now be written as a sum of one-electron Hamiltonians which can be solved separately, since the electrons can be assumed not to interact so long as the density is correct. This approximation is known as the Kohn-Sham approach[21,22] and greatly simplifies the problem of finding approximate solutions to the many-electron Schrödinger equation. The Kohn-Sham method is depicted in **Figure 2.**

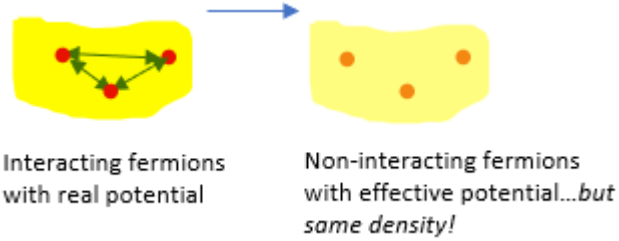

Figure 2: Visual representation of Kohn-Sham approximation.

All components of the DFT Hamiltonian are functionals of the electron density ρ, which, unlike wavefunctions, is an observable whose value can be confirmed experimentally. Specifically, DFT is considered a semi-empirical method which uses data for an idealized electron gas[23], approximated experimentally by generation of an electron gas from noble gas samples, in its estimate of the density

of a system. The energy of the system is expressed as

$$E(\rho) = T(\rho) + E_{Coul}(\rho) + E_{ext}(\rho) + E_{xc}(\rho) \quad (1)$$

Where

$$\text{Kinetic Energy, } T(\rho) = -\frac{1}{2}\sum_{i=1}^{N_{el}} \langle\varphi_i|\nabla^2|\varphi_i\rangle \quad (2)$$

$$\text{Electron-Electron Repulsion, } E_{Coul}(\rho) = \int \frac{\rho(r_1)\rho(r_2)}{r_1 - r_2} dr_1 dr_2 \quad (3)$$

$$\text{Electron-Nuclear Attraction, } E_{Ext}(\rho) = \int \sum_{A}^{Nuc} \frac{Z_A e^2}{r - R_A} \rho(r) dr \quad (4)$$

$$\text{Exchange-Correlation Potential, } E_{xc}(\rho) = \int V_{xc}(\rho, r)\rho(r) dr \quad (5)$$

When solved separately for each electron, each of these expressions can be solved exactly except the exchange-correlation potential. This term accounts for exchange between electrons of the same spin and the correlated motion of all electrons in the system. Even if an exact solution could be found, it would be so complicated that no amount of computational power could calculate it within a reasonable time frame[22]. Therefore, it is necessary to select an appropriate approximation for the exchange-correlation term – and there are very many to choose from. (The exact method chosen for this experiment and the rationale for doing so are discussed in SET OF FUNCTIONALS SELECTED). Note that all terms are functionals of the density except for the kinetic energy expression, for which exact solutions already exist before the Kohn-Sham approximation is applied.

The sum of the energy terms defined above is solved separately for each electron, then the one-electron energies are summed over the entire system. Therefore, we have an effective Hamiltonian which is simply a sum of one-electron Hamiltonians:

$$\widehat{H}_{eff} = \sum_{i=1}^{N\ electrons} \hat{h}_i(r_i) \quad (6)$$

Since the electron density is the square of the single-electron wavefunction φ_i, there is an added requirement that the density used for the energy calculations fits the requirement

$$\left[-\frac{1}{2}\nabla^2 + V_{Coul}(\rho, r) + V_{Ext}(\rho, r) + V_{xc}(\rho, r)\right]\varphi_i = \varepsilon_i \varphi_i \quad (7)$$

Pursuit of the ideal φ is aided by the idea that wavefunctions form a complete set, that is, as long as a set of wavefunctions are solutions to the Schrödinger equation, then any linear combination of them is also a solution. This validates the approach of adding more atomic orbital wavefunctions to find the combination that minimizes the overall energy. According to Variational Principle, an energy calculated in this way will always be equal to or higher than the true energy of the system. DFT methods follow this principle, so Variational Method is used to calculate the energy which most closely matches the true energy of the system. In Variational Hohenberg-Kohn method, the ground-state energy $E(\rho)$ and the electron density ρ are determined by constrained energy minimization of $E(\rho)$ using a LaGrange multiplier. The purpose of the LaGrange multiplier is to maximize or minimize a multivariable function subject to a constraint. The LaGrange multiplier is a new variable, μ, which is introduced as a coefficient to the constraint function. This product is used to define a new function, *L*,

which takes the form [(function to be minimized) – μ*(constraint) – c)], where c is a constant which the constraint expression is equal to. In the case of minimization of E(ρ), the constraint is the requirement that the integrand of the density over the entire volume of the system is equal to the number of electrons in the system (that is, $N_{el} = \int_V \rho(r)dr$). The LaGrangian is then[20]

$$L(\rho, r, \mu) = E(\rho) - [\mu(N_{el} - \int_V \rho(r)dr)] \quad (8)$$

From here, the gradient of *L* is set equal to zero and *L* is differentiated in turn with respect to each variable. $\frac{\delta L}{\delta \rho} = \frac{\delta L}{\delta r} = \frac{\delta L}{\delta \mu} = 0$ then yields a system of equations which can be solved with the help of a software package. The solutions to this system of equations are the minimum possible energy and density which meet the constraint requirements. By Variational Principle, the minimum energy is equal to, or slightly above, the actual energy of the system.

It is important to emphasize that DFT is a Self-Consistent Field (SCF) procedure, meaning the program makes an initial guess of which combination of atomic orbitals will minimize the total energy, then continues to add atomic orbitals until successive calculations yield the same energy. It is thus imperative that the correct set of functionals is selected from the start, given the high amount computational power and time required for the results of a DFT calculation to become self-consistent. For this reason, only the S_1 and much-contested S_4 state energies are to be calculated in this experiment, and cofactors that do not directly affect comparison of the hydrogen-bonding schemes (e.g. Cl^-, $Y_ZO\cdot$) are not included.

SET OF FUNCTIONALS SELECTED

While Becke's 3-Parameter Exchange Functional with Lee-Yang-Parr Correlation Corrections (B3LYP) has become widely popular for calculating the properties of inorganic complexes[24], the Minnesota 2006 (M06) suite of density functionals is preferred for calculations on compounds involving significant van der Waals interactions. Considering the emphasis on hydrogen bond arrangement in this experiment, M06 was selected as the method most suited to this study. While M06-2X gives the most accurate results, it only does so for nonmetals[25], so M06L is a better choice for the OEC. The advantage of M06L over other functionals in systems where weak forces are significant is that M06L incorporates van der Waals interactions into the exchange-correlation functionals themselves, while other methods simply treat these interactions as a perturbation.

The exchange and correlation functionals are, respectively,

$$E_x = \sum_\sigma \int [F_{X\sigma}^{PBE}(\rho_\sigma, \nabla\rho_\sigma) * f(w_\sigma) + \varepsilon_{X\sigma}^{LSDA} h_X(x_\sigma, z_\sigma)]dr \quad (9)$$

$$E_c = \int e_{\alpha\beta}^{UEG}[g_{\alpha\beta}(x_\sigma, x_\sigma) + h_{\alpha\beta}(x_{\alpha\beta}, z_{\alpha\beta})]\mathrm{dr} \quad (10)$$

The full derivation of these equations and each of their terms is a publication in itself (vz. Ref. 25), but certain elements of their form deserve comment. First, note that a new variable, spin (σ) has been introduced, with the related spin density (analogous to the relationship between the wavefunction and the electron density). The intrinsic adjustment for weak forces in the M06L functional has its origin in these terms. The large-scale rationale for their general forms is reasonable as well: exchange can happen between any two electrons in the system, so E_x is written as sum of integrals describing these processes, while E_c describes the correlated motion of all electrons in the system and is written as a

single integrand. Finally, note that these terms are composed of corrections. E_x is based off of the early Perdew-Burke-Ernzerhof exchange functional, corrected for the contribution of spin to the kinetic energy ($f(w_\sigma)$) and combined with the Local Spin Density Approximation exchange functional (itself the simplest density functional, dependent only on the spin density at a given point). The e, g, and h terms in the correlation functionals are also lengthy corrections with long derivation histories, but it is important to recognize that these terms are spin functions where electron spins are paired (hence the αβ nomenclature). The correlation functional takes an entirely different form when spins are paired. These are extremely abstract functions and the physical meaning behind them is beyond the scope of this rationalization. The crucial element for the reader to take away from this section is that M06L was chosen based on its unique ability to yield highly accurate results for systems where hydrogen-bonding and other weak forces are significant.

Computational Details

All comparative energy calculations were arranged for Q-Chem 5.1 package, DFT_D option (Dispersion-Corrected Density Functional Theory, D3 subset). Dispersion-Corrected DFT is used in conjunction with functionals like M06L which take dispersive forces into consideration; DFT-D3 is the most recent of these options. Although the exact inputs of the computation are rarely included in theoretical & computational chemistry publications, they are provided here due to their relevance to the independent research portion of the capstone project. Geometric units are in Ångstroms are are given in Cartesian coordinates in relation to the Mn^{II} atom in the S_1 state (Mn^V atom in the S_4 state). (Source: Q-Chem 5.1 User Manual[26]).

$comment

Single-point energy calculation for S1 state of Oxygen Evolving Complex proposed by Vrettos et al.

$end

$molecule

Mn	0.00	0.00	0.00
O	2.40	0.00	0.26
O	0.40	0.00	-2.06
Mn	2.40	0.00	-2.40
Ca	2.40	2.70	0.26
O	4.60	1.84	0.00
Mn	5.00	0.00	0.00
O	2.60	0.00	-2.10
O	2.50	1.90	-2.00
Mn	4.60	2.06	-2.32
O	1.68	0.00	0.00
O	0.10	2.70	0.26
H	1.05	0.00	0.00
H	-0.85	0.00	0.00

$end

$rem

JOBTYPE sp
EXCHANGE M06L
BASIS M06L
DFT_D
D3
[variable commands]

$end

$comment

Single-point energy calculation for proposed S1 state

$end

$molecule

Mn	0.00	0.00	0.00
O	2.40	0.00	0.26
O	0.40	0.00	-2.06
Mn	2.40	0.00	-2.40
Ca	2.40	2.70	0.26
O	4.60	1.84	0.00
Mn	5.00	0.00	0.00
O	2.60	0.00	-2.10
O	2.50	1.90	-2.00
Mn	4.60	2.06	-2.32
O	1.68	0.00	0.00
O	0.08	2.44	0.00
H	0.59	1.85	0.00
H	0.59	2.91	-1.06

$end

$rem
JOBTYPE sp
EXCHANGE M06L
BASIS M06L
DFT_D
D3
[variable commands]

$end

$comment

Single-point energy calculation for S4 state proposed by Vrettos et al.

$end

$molecule

Mn	0.00	0.00	0.00
O	2.40	0.00	0.26
O	0.40	0.00	-2.06
Mn	2.40	0.00	-2.40
Ca	2.40	2.70	0.26
O	4.60	1.84	0.00
Mn	5.00	0.00	0.00
O	2.60	0.00	-2.10
O	2.50	1.90	-2.00
Mn	4.60	2.06	-2.32
O	-2.2	0.00	0.00
H	0.00	0.95	0.00
H	0.92	-0.23	0.00

$end

$rem
JOBTYPE sp
EXCHANGE M06L
BASIS M06L
DFT_D
D3
[variable commands]

$end

$comment

Single-point energy calculation for proposed S4 state.

$end

$molecule

Mn	0.00	0.00	0.00
O	2.40	0.00	0.26
O	0.40	0.00	-2.06
Mn	2.40	0.00	-2.40
Ca	2.40	2.70	0.26
O	4.60	1.84	0.00
Mn	5.00	0.00	0.00
O	2.60	0.00	-2.10
O	2.50	1.90	-2.00

Mn	4.60	2.06	-2.32
O	0.00	2.20	0.00
H	0.08	2.13	0.10
H	0.00	2.20	-0.95

$end

$rem
JOBTYPE sp
EXCHANGE M06L
BASIS M06L
DFT_D
D3
[variable commands]

$end

Discussion/Implications

None of the recently proposed mechanisms reviewed in this study are prohibitive – structurally or mechanistically – of hydrogen-bonding between the protons on the substrate waters and the μ-oxo bridges of the Oxygen Evolving Complex of Photosystem II. Although some of the synthetic analogues studied by the Dismukes, Hillier, Limburg, Pecoraro, and Dau groups does not favor this hydrogen-bonding arrangement at one or more S-states, it may be reasonably argued that these are synthetic analogues which lack the structural features of the real OEC that permit such interactions to begin with. The hydrogen-bonding scheme proposed in this paper is then applicable regardless of the mechanism selected, although the mechanism set forth by Vrettos and colleagues was found to be the most reliable of those reviewed.

If the experiment proposed in this paper is eventually conducted by a funded research group and the proposed hydrogen-bonding scheme is found to lower the energy of the overall structure, then it is highly likely that hydrogen-bond stabilization by the bridging oxygens is a crucial feature of the mechanism of the OEC. If this is indeed the case, it strongly suggests that incorporation of such metal-oxygen-metal bridges in synthetic catalysts is the key to designing a *renewable energy catalyst whose only by-product is molecular oxygen*. It is my hope that a funded researcher will one day conduct this experiment and launch a new era of more efficient solar cell design.

References

[1] Ferreira, K.N.; Iverson, T.M.; Maghlaoui, K.; Barber, J.; Iwata, S. Architecture of the Photosynthetic Water Oxidation Center. *Science*, **2004**, 303, pp. 1831-1838.
[2] Umena, Y.; Kawakami, K.; Shen, J.R.; Kamiya, N. Crystal Structure of Oxygen-Evolving Photosystem II at a Resolution of 1.9 Å. *Nature*, **2011**, 473 (7345), pp. 55-60.
[3] Kok, B.; Forbrush, B.; McGloin, M. Cooperativity of Charges in Photosynthetic O_2 Evolution – I. A Linear Four-Step Mechanism. *Photochem. & Photobiol.* **1970**, 11(6), 457-75.
[4] Kretschmann, H.; Schlodder, E.; Witt, H.T. Net Charge Oscillation and Proton Release During Water Oxidation in Photosynthesis. An Electrochromic Band Shift Study at pH 5.5-7.0 *Biochim. Et Biophys. Acta*, **1996**, 1274 (1-2), pp. 1-8.
[5] Schlodder, E.; Witt, H.T. Stoichiometry of Proton Release from the Catalytic Center in Photosynthetic Water Oxidation – Reexamination by a Glass Electrode Study at pH 5.5-7. *J. Biol.*

Chem. **1999**, 274, 30387-30392.
[6] Tang, X-S.; Randall, D.W.; Force, D.A.; Diner, B.A.; Britt, R.D. Manganese-Tyrosine Interaction in the Photosystem II Oxygen-Evolving Complex. *J. Am. Chem. Soc.* **1996**, 118, 32, 7638-7639.
[7] Hillier, W.; Wydrzynski, T. The Affinities for the Two Substrate Water Binding Sites in the O_2 Evolving Complex of Photosystem II Vary Independently During S-State Turnover. *Biochemistry*, **2000**, 39 (15), pp. 4399-4405.
[8] Hendry, G.; Wydrzynski, T. ^{18}O Isotope Exchange Measurements Reveal that Calcium is Involved in the Binding of One Water-Substrate Molecule to the Oxygen-Evolving Complex in Photosystem II. *Biochemistry*, **2003**, 42 (20), pp. 6209-6217.
[9] Vrettos, S.; Limburg, J.; Brudvig, G. Mechanism of Photosynthetic Water Oxidation: Combining Biophysical Studies of Photosystem II with Inorganic Model Chemistry. *Biochim. Et Biophys. Acta – Bioenergetics*, **2001**, 1503, pp. 229-245.
[10] Vincent, J.B.; Christou, G. The Molecular 'Double-Pivot' Mechanism for Water Oxidation. *Inorg. Chim. Acta*, **1987**, 136, pp. L41.
[11] Ruettinger, W.; Yagi, M.; Wolf, K.; Bernasek, S.; Dismukes, G.C. O_2 Evolution from the Manganese-Oxo Cubane Core $Mn_4O_4^{6+}$: A Molecular Mimic of the Photosynthetic Water Oxidation Enzyme? *J. Am. Chem Soc.* **2000**, 122 (42), pp. 10353-10357.
[12] Petrie, S.; Stranger, R.; Pace, R.J. Location of Potential Substrate Water Binding Sites in the Water Oxidizing Complex of Photosystem II. *Angew. Chemie Intl. Ed.* **2010**, 49 (25), pp. 4233-4236.
[13] Loll, B.; Kern, J.; Saenger, W.; Zouni, A.; Biesiadka, J. Towards Complete Cofactor Arrangement in the 3.0 Å Resolution Structure of Photosystem II. *Nature*, **2005**, 483, pp. 1040-1044.
[14] Limburg, J.; Szalai, V.A.; Brudvig, G.W. A Mechanistic and Structural Model for the Formation and Reactivity of a MnV=O Species in Photosynthetic Water Oxidation.
[15] Pecoraro, V.L.; Baldwin, M.J.; Caudle, M.T.; Hsieh, W-Y. A Proposal for Water Oxidation in Photosystem II. *Pure & Applied Chem.* **1998**, 70(4), pp. 925-929.
[16] Dau, H.; Iuzzolino, L.; Dittmer, J. The Tetramanganese Complex of Photosystem II During its Redox Cycle – X-Ray Absorption Results and Mechanistic Implications. *Biochim. Biophys. Acta*, **2001**, 1503, pp. 24-29.
[17] Schaeffer & Laurent, "The Role of Functional Groups in Drug-Receptor Interactions" in *The Practice of Medicinal Chemistry*, 4th ed. **2008**.
[18] Cramer, C.J.; Truhlar, D.G. Density Functional Theory for Transition Metals and Transition Metal Chemistry. *Phys. Chem. Chem. Phys.* **2009**, 46, pp. 10741-11064.
[19] Hoganson, C.W.; Babcock, G.T. A Metalloradical Mechanism for the Generation of Oxygen from Water in Photosynthesis. *Science*, **1997**, 277 (5334), pp. 1953-1956.
[20] Franzen Lecture at NCSU, http://www4.ncsu.edu/~franzen/public_html/CH736/lecture/DFT_Method_H.pdf
*Please note that this and other www4 files from NC State are no longer available online.
[21] Lecture 6 by C-K. Skylaris at Southampton, "CHEM 6085: Density Functional Theory." May be viewed at https://www.southampton.ac.uk/assets/centresresearch/documents/compchem/DFT_L6.pdf
[22] Lecture "Introduction to Density Functional Theory" by C. David Sherrill, Georgia Institute of Technology. May be viewed at http://vergil.chemistry.gatech.edu/notes/DFT-intro.pdf
[23] Engel & Reid *Physical Chemistry* 3rd ed. **2005**.
[24] Sousa, S.F.; Fernandes, P.A.; Ramos, M.J. General Performance of Density Functionals. *J. Phys. Chem. A*. **2007**, 111, pp. 10439-10452.
[25] Zhao, Y.; Truhlar, D.G. The M06 Suite of Density Functionals for Main Group Thermochemistry, Thermochemical Kinetics, Noncovalent Interactions, Excited States, and Transition Elements: Two New Functionals and Systematic Testing of Four M06 Functionals and Twelve Other Functionals. *TCA (Mark S. Gordon Festschrift Issue)* **2007** (supplemental information).
[26] *Q-Chem 5.1 User's Manual*, available online at https://www.q-chem.com/qchem-

website/manual/qchem51_manual/index.html